CAROTENE CONCENTRATES from VEGETABLE LEAF WASTES

Certain vegetable leaf meals are rich sources of carotene, ranging in potency from 300 to 700 micrograms per gram. Petroleum ether solvents combine good solvent properties for carotene in leaf meals with relatively poor solvent properties for other plant pigments. A number of procedures for the preparation of carotene concentrates from vegetable leaf meals have been devised. Most of them are based on the rapid saponification of chlorophyll in petroleum ether solution, followed by adsorption treatment with hydrated lime. Upon removal of the solvent, deep red carotene concentrates equivalent to 20,000–200,000 I.U. vitamin A per gram are obtained. The yield of purified carotene is from 85 to 95% of the carotene in the original extract.

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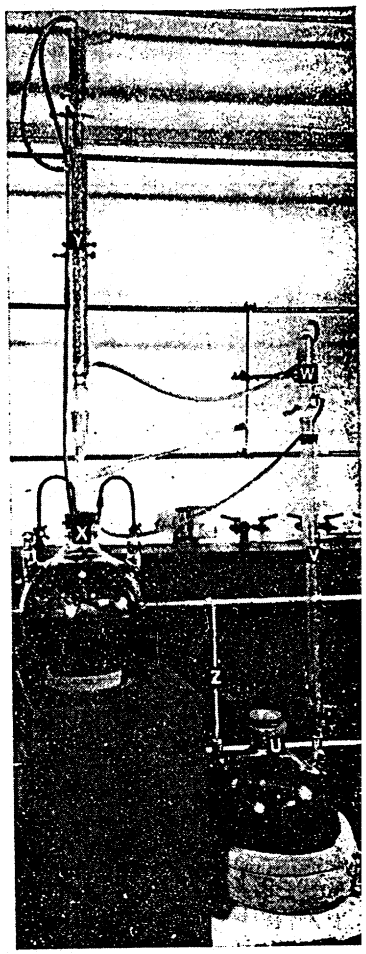

Figure 1. Large Soxhlet Extracto

U. 22-Liter Pyrex 3-neck flask, heated with Glas-col 22-liter hemispherical mantle or a internal steam coil

V. Condenser, used with either steam o cold water

W. Friedrich condenser (Pyrex 3440)

X. 3-Neck, Pyrex, 22-liter flask with tubu lature at bottom

Y. Several auxiliary condensers

Z. Siphon tube attached to flask *U* an tubulature of flask *X*

PREVIOUS reports from this Laboratory have shown that dry vegetable leaf wastes have a high carotene content (*2*, *3*). Although they may vary within wide limits, spinach, beet, carrot, turnip, kale, and broccoli leaf wastes, when properly collected and dried, have an average range of 300 to 700 micrograms of carotene per gram. Hence it is conceivable that carotene concentrates may be economically prepared from these materials. This paper describes and compares various procedures devised for preparing carotene concentrates from vegetable leaf wastes. The chief operations involved are extraction of carotene and purification of the extract.

The leaf wastes, obtained from local packing houses and farms, were trucked to the Laboratory, where they were either dried at once or stored for a few days at 4° C. After the wastes were dried in a through-circulation air dryer, the leaves were separated from the stems by screening according to the method of Kelley, Wall, and Willaman (*3*). The initial moisture content ranged from 80 to 90%; in most cases the final moisture content was 5%.

To determine carotene and xanthophyll, these pigments must be dissolved in petroleum ether. If they are dissolved in other solvents, the solvent must be removed under vacuum and the residue taken up in petroleum ether. An aliquot containing 50 to 500 micrograms each of carotene and xanthophyll was adsorbed on a mixture of 3 parts Hyflo Supercel and 1 part activated magnesia No. 2641. The carotene was separated from xanthophyll and chlorophyll by washing the adsorption column with a solution of 5% acetone in Skellysolve B, according to the method of Wall and Kelley (*11*). The xanthophyll was then removed from the column by washing with a solution of 20 to 30% acetone in Skellysolve B.

Chlorophyll was determined by direct reading in a photoelectric colorimeter, according to the method of Petering, Wolman, and Hibbard (*7*).

CAROTENE EXTRACTION

Extractions were made in a Soxhlet and percolation apparatus. Most of the large-scale extractions were carried out in a Soxhlet apparatus of 4.5-kg. (10-pound) capacity (Figure 1), which was similar in some respects to that described by Rose, Freeman, and McKinney (*8*). The material to be extracted was ground 3(to 40 mesh in a Wiley mill and put in bags holding 450 grams each. A 4.5-kg. charge was put into flask *X*. After the bags were soaked with solvent, 12 to 14 liters of solvent was poured into flask *U*. Steam was run through condenser *V*, and the extraction started. For rapid extraction, the solvent was boiled a such a rate that siphoning took place two or three times per hour Since considerable back pressure develops, the height of the si phon tube must be experimentally adjusted.

The apparatus was converted for distillation by removing th siphon tube and substituting a 22-liter tubulature flask, with th tubulature closed during distillation. For reflux operations, th siphon tube and condenser *W* were removed, an auxiliary reflu condenser was attached to condenser *V*, and cold water was ru through the condensers.

Carotene has been extracted from plant material with a larg number of organic solvents. The solvents tested here include

TABLE I. RATE OF EXTRACTION OF CAROTENE FROM BROCCOLI LEAF MEAL WITH VARIOUS SOLVENTS

Time, Hr.	% of Total Carotene Extracted				
	SK F	SK B	SK C	Acetone	Trichloroethylene
1	77.5	89.0	90.5	83.0	93.0
2	86.0	95:0	93.0	91.5	94.0
3	90.0	100.0	95.0	100.0	99.5
4	100.0	100.0	97.5	100.0	98.0
5	100.0	100.0	100.0	100.0	100.0

TABLE II. PIGMENT COMPOSITION[a] OF BROCCOLI LEAF MEAL EXTRACTS PREPARED WITH VARIOUS SOLVENTS

Solvent	Carotene, %	Xanthophyll, %	Chlorophyll, %	Ratio, Carotene to: Xanthophyll	Ratio, Carotene to: Chlorophyll
SK F	18.1	24.4	57.5	0.74	0.31
SK B	15.2	25.6	59.2	0.59	0.26
SK C	10.8	28.6	60.6	0.38	0.18
Acetone	4.8	6.4	88.8	0.75	0.05
Trichloroethylene	5.0	15.2	79.8	0.33	0.06

[a] Only carotene, xanthophyll, and chlorophyll are considered in this table.

Skellysolve F (b.p. 35° to 59° C., primarily pentane), Skellysolve B (b.p. 65° to 70° C., primarily hexane), Skellysolve C (b.p. 88° to 98° C., primarily heptane), acetone, chloroform, trichloroethylene, and carbon tetrachloride. All extracted large proportions of other plant pigments and lipoids along with the carotene. The amount and type of these contaminants varied widely, depending on the solvent used.

The rate of extraction of carotene from broccoli leaf meal by different solvents was determined in a Soxhlet apparatus of 1-pound capacity. The results are shown in Table I. All the solvents extracted 100% of the carotene in 4 to 5 hours. Skellysolves B and C and trichloroethylene extracted approximately 90% in 1 hour; Skellysolve F and acetone extracted it somewhat less rapidly.

The quantity of xanthophyll and chlorophyll extracted by the various solvents was determined in the 5-hour extracts. The results are shown in Table II. The pigment composition of the extracts varied widely, depending on the solvent used. Since all the extracts had approximately the same total carotene content, the differences in percentages of these pigments in Table II are due primarily to variations in xanthophyll and chlorophyll extracted by the different solvents; chlorophyll showed the greatest variation. Skellysolve F extracted about three times as much chlorophyll as carotene; the others extracted increasing amounts until approximately twenty times as much chlorophyll as carotene was obtained with acetone and trichloroethylene.

It has been reported that petroleum ether will not extract either chlorophyll or xanthophyll from plant material (*9*, *12*). With the various leaf meals tested, appreciable quantities of chlorophyll and xanthophyll were extracted with a wide range of petroleum ethers. Although pure xanthophyll and chlorophyll are only slightly soluble in low-boiling petroleum ether, the solubilities of these compounds seem to be markedly affected by other fat-soluble substances in plant material.

PURIFICATION OF EXTRACTS BY DIRECT ADSORPTION

Since there is relatively little demand for crystalline carotene, efforts were centered on preparing a concentrate of carotene in the natural plant lipoids. Skellysolve B, which has many advantages from the standpoint of both purity of the extract and cost, was used for most of the large-scale extractions. A 4.5-kg. batch of leaf meal was extracted in the large Soxhlet apparatus, and the extract concentrated to 3 or 4 liters. Little or no loss of carotene occurred even when the concentration took place at atmospheric pressure. The concentrated extract was used for the purification experiments.

This procedure was based on the use of activated magnesia No. 2641 (Westvaco Chlorine Products Company), extensively employed by Strain (*10*) as an adsorbent for separation of carotenoid mixtures and also used by Wall and Kelley (*11*) in the determination of carotene in leaf tissue.

Attempts to purify concentrated plant extracts in large chromatographic columns composed of Hiflo Supercel–activated magnesia mixtures were not successful, since the chlorophyll spread through practically the whole length of the column before carotene separated.

When the extract was stirred directly with magnesia, the slurry could be filtered in relatively shallow layers on large Büchner funnels without a filter aid. The carotene was then washed off the adsorbent with pure Skellysolve B or 5% acetone in Skellysolve B. The clear red extract obtained was free of chlorophyll and had little or no xanthophyll. From 0.20 to 0.45 kg. of magnesia was required for every kg. of leaf meal extracted, depending on the source of the extract. The adsorbent was thoroughly stirred with the extract and filtered on a large Büchner funnel, and the cake was well broken and washed three or four times with a total of 6 to 8 liters of Skellysolve B. The yield of carotene averaged about 85% of the quantity initially present. The solvent was removed *in vacuum*, leaving a deep-red oil which solidified on cooling. From 0.45 to 0.56 gram of carotene in 30 to 45 grams of plant lipoids was obtained per kg. of broccoli leaf meal.

PURIFICATION BY SAPONIFICATION OF CHLOROPHYLL

The amount of adsorbent could be greatly reduced if the chlorophyll could be removed from the extract before adsorption. Although chlorophyll may be separated from carotene by direct saponification of the plant material, according to the method of Holmes and Leicester (*1*) or by procedures based on the classical Willstätter-Stoll procedure (*12*), such methods are time consuming and do not lend themselves to commercial practice. Therefore, saponification of chlorophyll was attempted in the petroleum ether extract.

As a result of these experiments a rapid and simple method of removing chlorophyll from plant extracts was developed. In certain respects the method is similar to the analytical procedure of Kuhn and Brockmann (*4*). It was found that 95% ethanol containing 5% potassium hydroxide or saturated with sodium hydroxide was completely miscible with petroleum ether, and when a petroleum ether plant extract was mixed with the alcoholic potassium hydroxide and boiled, most of the chlorophyll was rapidly saponified.

To determine optimum conditions, a study was made of the effect of alkali concentration, length of heating period, and ratio of alcohol to petroleum ether. Concentrations of alkali used were 1, 5, and 10% potassium hydroxide in 95% ethanol; the time of boiling was 0.5, 1, and 2 hours; and the ratio of alcohol to Skellysolve B ranged from 1:4 to 1:1. All combinations of these factors, with the exception of 1% potassium hydroxide, yielded practically the same results. In most cases 97 to 99% of the total chlorophyll was saponified, which left a residual chlorophyll amounting to 10 to 5%, respectively, of the total pigment mixture in the petroleum ether extract. The combination of 5% potassium hydroxide or saturated solution of sodium hydroxide in 95% ethanol, 0.5-hour reflux time, and a 1:4 ratio of alcohol to Skellysolve B was selected as most economical and effective.

The following method was thus evolved: The concentrated Skellysolve B extract (3 to 4 liters) from a 4.5-kg. batch of leaf meal is mixed with 1 liter of 95% ethanol containing 5% potassium hydroxide. The mixture is vigorously refluxed for 0.5 hour and then cooled. Sufficient water (187.5 ml.) is added to make the final alcohol concentration 80%. The extract is shaken and then allowed to stand about 15 minutes. Two layers are formed. The lower consists of the aqueous alcohol with dissolved saponification products and also some xanthophyll; the upper consists of the Skellysolve B extract, from which most of

TABLE III. EFFECT OF SAPONIFICATION ON CHLOROPHYLL IN PETROLEUM ETHER EXTRACT OF BROCCOLI LEAF MEAL

Conditions after:	Mg. per Kg. of Leaf Meal	% of Original Chlorophyll	% of Total Pigment
Original extraction	2640	100.0	74.5
First saponification	111	4.2	15.3
Continuous 80% ethano extraction	43	1.6	10.5
Second saponification	34	1.3	7.2
Third saponification	34	1.3	7.2

TABLE IV. RECOVERY AND PURIFICATION OF CAROTENE FROM PETROLEUM ETHER EXTRACT OF MIXED VEGETABLE LEAF MEAL

Conditions after:	Carotene		Xanthophyll		Chlorophyll	
	Mg./kg. leaf meal	% of total pigment	Mg./kg. leaf meal	% of total pigment	Mg./kg. leaf meal	% of total pigment
Original extn.	240	17.0	162	11.5	1010	71.5
Saponification	226	63.5	90	25.0	39	11.5
Adsorption on magnesia, 400 g.	220	100.0	0	0.0	0	0.0

TABLE V. EFFICIENCY OF ADSORBENTS IN PURIFYING A SAPONIFIED PLANT EXTRACT IN SKELLYSOLVE B

Adsorbent, Grams	% Carotene in Total Pigment	G. Adsorbent/G. Activated Magnesia
Activated magnesia, 200	73.6	1.0
Hydrated lime[a], 1200	67.0	6.0
Lime, 1200	66.7	6.0
Magnesium carbonate, 800	77.0	4.0
Calcium carbonate, 2400	63.0	12.0
Bauxite, 1200	69.0	6.0

[a] Through courtesy of the Warner Company, we recently received specially prepared, dolomitic hydrated limes which were active adsorbents. These lime preparations have an adsorption ratio of 2.0, or half the adsorptive capacity of activated magnesia No. 2641.

the chlorophyll and some xanthophyll have been removed. The aqueous alcohol layer contains little carotene, and therefore it is discarded for recovery of the alcohol. A 6-liter separatory funnel, well illuminated, is used to separate the two layers.

At this stage the Skellysolve B extract is usually dark brown, probably owing to small amounts of unsaponified chlorophyll remaining in solution. It is wet and contains traces of alkali, but the subsequent treatment renders any drying or removal of alkali unnecessary.

Table III shows the percentage of chlorophyll remaining after saponification of an extract from 4.5 kg. of broccoli leaf meal. The saponified extract was subjected to a continuous 80% ethanol extraction, after which the extract was twice resaponified. The first saponification removed about 96% of the chlorophyll; subsequent treatment with alcohol removed an additional 2 to 3%, which was probably emulsified in the petroleum ether solvent. Two further saponifications failed to remove the remaining 1%. This remnant probably was a decomposition product. All the extracts obtained by the various treatments were dark brown, owing to the fact that the remaining 1% of chlorophyll or its decomposition products constituted from 5 to 10% of the total pigment.

PURIFICATION BY SAPONIFICATION AND ADSORPTION

Although the extract, after saponification and separation of saponification products, can be used to make a crude concentrate, for many purposes a purer extract is desirable. The saponified petroleum ether solution is stirred with 200 to 400 grams of activated magnesia No. 2641 and filtered on a large Büchner funnel, after which the adsorbent is washed three times with a total of 3 to 6 liters of Skellysolve B. A deep red carotene preparation, free of water and alkali, results. Recovery and purification of carotene in a typical experiment are shown in Table IV. This procedure results in a carotene solution free of chlorophyll and xanthophyll. Carotene yields of 85 to 95% have been consistently obtained.

Although the saponification procedure greatly reduced the quantity of magnesia required for purification, the cost of the magnesia was still too high for commercial use. A number of less expensive materials were therefore tested, including technical calcium oxide, technical calcium hydroxide, calcium carbonate, *sec*-calcium phosphate, magnesium oxide, magnesium carbonate, *sec*-magnesium phosphate, activated alumina, aluminum oxide, and certain grades of bauxite (a natural aluminum oxide). Acidic compounds, extremely alkaline compounds, and various carbon blacks destroy carotene or adsorb it so firmly that it can not be removed.

The relative efficiency of the adsorbents was determined by stirring aliquots of a saponified extract with increasing increments of adsorbent. The percentage of carotene in the total pigment mixture was determined after each increment of adsorbent. Using the results obtained with activated magnesia as a standard, the weights of the various adsorbents required to give approximately the same degree of purification obtained with activated magnesia were noted. The relative efficiency of the adsorbent could then be determined from the ratio of grams of adsorbent to grams of activated magnesia. Some typical results are shown in Table V. The adsorbents tested can be divided into three groups in order of decreasing efficiency: (1) activated magnesia No. 2641, activated alumina; (2) CaO, $Ca(OH)_2$, $MgCO_3$, Mg(bauxite; (3) $CaCO_3$, $CaHPO_4$, $MgHPO_4$.

As a result of these tests, technical hydrated lime was selected for large-scale experiments. It is readily available and although it is only about one sixth as active as activated magnesia, it costs only about one fiftieth as much. The following example illustrates the use of hydrated lime: A 4-liter concentrate of a saponified extract of 4.5 kg. of leaf meal was thoroughly stirred with 1.0 to 2.0 kg. of commercial hydrated lime and filtered. The filter cake was thoroughly washed three or four times with 6 to liters of Skellysolve B. A deep red solution was obtained in which 70 to 80% of the total pigment was carotene and 30 20% xanthophyll. The yield of carotene was from 85 to 95 of that originally present in the crude extract.

A number of commercial hydrated limes were tested. removed the remaining chlorophyll and variable proportions xanthophyll from the saponified extracts. It is not practical remove all the xanthophyll since an inordinately large bulk lime is required. Moreover, the product can be made xanthophyll-free economically by further treatment with 80 to 8 ethanol. For many purposes the xanthophyll in the extra is not objectionable.

Saponification followed by lime adsorption removed ab half the plant lipoids in a Skellysolve B extract of broccoli meal. About 0.45–0.56 gram of carotene in 20 grams of pl lipoids was obtained per kg. of meal.

CHOICE OF SOLVENTS

Similar experiments were conducted with Skellysolves F C. Saponification of chlorophyll proceeded equally well, in general greater adsorption was obtained in Skellysolve F t in the other petroleum ether solvents. Skellysolve F extra less xanthophyll and chlorophyll from plant materials t Skellysolves B or C, and a pure carotene extract was obtained less adsorbent. On the other hand, Skellysolve F has a low ing point, and consequently solvent losses might be high.

Since the use of petroleum ether solvents is attended with and explosion hazards, experiments were conducted with ch nated solvents, particularly trichloroethylene and carbon t chloride. These solvents extracted about three to four tim much chlorophyll in two to three times as much xantho from plant material as did the petroleum ether solvents. saponification reaction with these solvents gave variable re (With these chlorinated solvents the upper layer is aqueo cohol.) In both cases about 90 to 95% of the chlorophyl saponified. There was little loss of carotene when tricl ethylene was used but great loss with carbon tetrachloride,

TABLE VI. RECOVERY AND PURIFICATION OF CAROTENE OBTAINED FROM A TRICHLOROETHYLENE EXTRACT OF BROCCOLI LEAF MEAL

Conditions after:	Carotene Mg./kg. leaf meal	Carotene % of total pigment	Xanthophyll Mg./kg. leaf meal	Xanthophyll % of total pigment	Chlorophyll Mg./kg. leaf meal	Chlorophyll % of total pigment
Original extn.	570	4.8	1160	9.6	10,350	85.6
Saponification	530	25.0	955	45.4	625	29.6
Adsorption on hydrated lime, 6000 g.	489	35.0	910	65.0	0	0

TABLE VII. PIGMENT COMPOSITION OF EXTRACTS PREPARED FROM BROCCOLI LEAF MEAL BY VARIOUS METHODS

Method	Color	Carotene, %[a]	Xantho-phyll, %[a]	Chloro-phyll, %[a]	Vitamin A[b], I.U./G. Solids
1. Petroleum ether extn. and saponification	Green-brown	50–60	20–30	10–20	30,000–40,000
2. Same as 1, plus lime treatment	Red	70–80	20–30	0	30,000–40,000
3. Same as 1, plus 80% ethanol extn.	Brown	80–90	5–10	5–10	30,000–40,000
4. Same as 2, plus 80% ethanol extn.	Red	100	0	0	30,000–40,000
5. Same as 2, plus removal of fats from SK B soln. by chilling	Red	95–100	0–5	0	160,000–180,000
6. Same as 5, plus adsorption on 1:1 activated magnesia (Hi-flo Supercel)	Red	100	0	0	200,000–220,000
7. Crystallisation of extract 6	Red	100	0	0	1,660,000

[a] On basis of total pigment.
[b] Values obtained by multiplying micrograms of carotene found by chemical analysis by conventional conversion factor 1.66.

was undoubtedly due to decomposition of the solvent in alcoholic potassium hydroxide. There is risk of such decomposition with all chlorinated solvents commonly used.

The quantity and purification of carotene obtained from a trichloroethylene extract of 4.5 kg. of broccoli leaf meal are shown in Table VI. After adsorption the solution was deep red; 35% of the total pigment consisted of carotene and 65% of xanthophyll. The yield of carotene was 86% of that in the original extract. Little xanthophyll was removed in the saponification procedure because it was necessary to dilute the ethanol 50% with water to separate the trichloroethylene and alcohol layers. Practically no xanthophyll was removed by adsorption on hydrated lime. If desired, the xanthophyll left in the lime-treated extract could be removed with aqueous alcohol.

Chlorinated solvents are relatively expensive, and large amounts of adsorbent are required for purification. On the other hand, the cost of equipment for these solvents would be lower, owing to the greatly reduced fire hazard.

In other experiments the leaf meal was extracted with trichloroethylene, which was removed in vacuum, and the residue taken up in a much smaller amount of Skellysolve B. No loss of carotene occurred. In this procedure there is the advantage of conducting the large volume phase of the process with a nonflammable solvent. The carotene can then be purified much more efficiently in petroleum ether.

LIQUID-LIQUID PURIFICATION

Use of aqueous ethanol in purifying the leaf meal extracts was studied. A continuous extractor of a type used for extracting light liquids with heavier ones was devised for this purpose. Aqueous ethanol of approximately 60% concentration was boiled, and the vapors (80 to 85% ethanol) were condensed, passed through a tall column of petroleum ether extract, and returned to the boiling flask. When a saponified Skellysolve B solution was thus extracted, a solution was obtained in which 85 to 90% of the total pigment was carotene and the remainder was xanthophyll and unsaponified chlorophyll. There was little loss of carotene in this operation. The extract was dark brown, as contrasted with the clear, deep red extracts obtained by the adsorption technique. Such a carotene concentrate may have uses for which the color is not objectionable.

Purification by layering was also investigated. Petroleum ether extracts were shaken in separatory funnels with 80 to 85% ethanol. Various ratios of alcohol to petroleum ether were tested. The higher the ratio of alcohol to petroleum ether, the more rapid the removal of alcohol-soluble impurities. As in the liquid-liquid extractor, a dark brown carotene extract was obtained in which 85 to 90% of the total pigment was carotene.

Similarly, extracts containing xanthophyll but no chlorophyll after adsorption were purified by the alcohol treatment. Such a procedure is practical only when a pure carotene extract is desired.

ACETONE EXTRACTION

The procedures described for extraction and purification of carotene were compared with the methods of Petering *et al.* (*5*, *6*) originally described for use with alfalfa leaf meal. Carotene yields of 68–89% were secured, the lower results with the use of technical barium hydroxide and the higher with C.P. grade. The acetone–barium hydroxide procedure not only resulted in lower yields of carotene when technical reagents were used, but also required more steps and somewhat more expensive chemicals than the petroleum ether extraction and saponification procedure.

POSSIBLE CAROTENE PRODUCTS

Carotene concentrates of varying degrees of purity may be prepared for specific markets by the procedures outlined. Table VII summarizes the methods used and the pigment composition of the extracts prepared by the various methods.

The product obtained in method 1 by saponification only was a crude extract, partly purified in respect to xanthophyll and chlorophyll. The extract freed of solvent might be suitable for a crude animal feed supplement. When this extract was treated with lime (method 2), a deep red concentrate free of chlorophyll was formed. This concentrate contained 18,000 to 24,000 micrograms of carotene (30,000 to 40,000 International Units of vitamin A) per gram of concentrate. It was easily soluble in vegetable oils, and might be suitable for a food or feed supplement. Treating the saponified extract with 80 to 85% ethanol or 90% methanol produced a concentrate which contained relatively less pigment impurities than the concentrate from method 2. Owing to the presence of a small amount of chlorophyll or its degradation products, this concentrate was brown instead of the red color of a typical carotene concentrate. It would probably be acceptable where an off-color was not objectionable. The concentrate from method 4, resulting from the further treatment of that from method 2 with 80 to 85% ethanol or 90% methanol, yielded a carotene preparation free of xanthophyll and chlorophyll. The concentrates from methods 1 to 4 had about the same provitamin A content, 18,000 to 24,000 micrograms (30,000 to 40,000 I.U. of vitamin A) per gram. When the concentrate from method 2 was dissolved in petroleum ether and chilled, a copious precipitate of various lipoidal substances was obtained. Most of the carotene remained in solution and was filtered off. In this way high-potency concentrates containing 97,000 to 108,000 micrograms of carotene (160,000 to 180,000 I. U. of vitamin A) per gram of solids were obtained. Further treatment of this concentrate with a mixture of activated magnesia and Hiflo Supercel removed some impurities, and the resulting oil contained 120,000 to 134,000 micrograms of carotene (200,000 to 222,000 I.U. of vitamin A) per gram. Crystalline carotene (melting at 171° to 173° C., corrected) was obtained from the concentrates from methods 5 and 6 by low-temperature crystallization.

Methods presented in this paper for the preparation of carotene concentrates will be tested in the pilot plant to de-

termine their suitability for large-scale operation and to obtain data on the cost of the various steps.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Raymond T. Merrow, Samuel Krulick, and Reba Baum in various phases of this research.

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